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<p>(21) International Application Number: PCT/US98/07178 (22) International Filing Date: 9 April 1998 (09.04.98) (30) Priority Data: 08/831,570 9 April 1997 (09.04.97) US (71) Applicant (for all designated States except US): RHONE-POULENC AGRO [FR/FR]; Dépt. Pro- priété Industrielle, 14-20, rue Pierre Balzet, F-69009 Lyon (FR). (72) Inventors; and (75) Inventors/Applicants (for US only): THOMAS, Terry, L. [US/US]; 2804 Cloister Drive, College Station, TX 77845 (US). BEREMAND, Phillip, D. [US/US]; 9208 Brookwa- ter Circle, College Station, TX 77845 (US). NUNBERG, Andrew, N. [US/US]; 12215-B Encanto Lane, Maryland Heights, MO 63043 (US). (74) Agents: DiGIGLIO, Frank, S. et al.; Scully, Scott, Murphy & Presser, 400 Garden City Plaza, Garden City, NY 11530 (US).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p>(54) Title: A SUNFLOWER ALBUMIN 5' REGULATORY REGION FOR THE MODIFICATION OF PLANT SEED LIPID COMPOSITION (57) Abstract <p>The present invention is directed to 5' regulatory regions of a sunflower albumin gene. The 5' regulatory regions, when operably linked to either the coding sequence of a heterologous gene or a sequence complementary to a native plant gene direct expression of the coding sequence or complementary sequence in a plant seed. The regulatory regions are useful in expression cassettes and expression vectors for the transformation of plants. Also provided are methods of modulating the levels of a heterologous gene or native plant gene such as a fatty acid synthesis or lipid metabolism gene by transforming a plant with the subject expression cassettes and expression vectors.</p></p>		

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1 A SUNFLOWER ALBUMIN 5' REGULATORY REGION FOR THE
 MODIFICATION OF PLANT SEED LIPID COMPOSITION

BACKGROUND OF THE INVENTION

5 Seed oil content has traditionally been
modified by plant breeding. The use of recombinant
DNA technology to alter seed oil composition can
accelerate this process and in some cases alter seed
10 oils in a way that cannot be accomplished by breeding
alone. The oil composition of *Brassica* has been
significantly altered by modifying the expression of a
number of lipid metabolism genes. Such manipulations
of seed oil composition have focused on altering the
proportion of endogenous component fatty acids. For
15 example, antisense repression of the $\Delta 12$ -desaturase
gene in transgenic rapeseed has resulted in an
increase in oleic acid of up to 83%. Topfer et al.
1985 *Science* 268:681-686.

 There have been some successful attempts at
20 modifying the composition of seed oil in transgenic
plants by introducing new genes that allow the
production of a fatty acid that the host plants were
not previously capable of synthesizing. Van de Loo,
et al. (1995 *Proc. Natl. Acad. Sci USA* 92:6743-6747)
25 have been able to introduce a $\Delta 12$ -hydroxylase gene
into transgenic tobacco, resulting in the introduction
of a novel fatty acid, ricinoleic acid, into its seed
oil. The reported accumulation was modest from plants
carrying constructs in which transcription of the
30 hydroxylase gene was under the control of the

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cauliflower mosaic virus (CaMV) 35S promoter.

1 Similarly, tobacco plants have been engineered to
produce low levels of petroselinic acid by expression
of an acyl-ACP desaturase from coriander (Cahoon et
al. 1992 *Proc. Natl. Acad. Sci USA* 89:11184-11188).

5 The long chain fatty acids (C18 and larger),
have significant economic value both as nutritionally
and medically important foods and as industrial
commodities (Ohlrogge, J.B. 1994 *Plant Physiol.*
104:821-826). Linoleic (18:2 $\Delta^9,12$) and α -linolenic
10 acid (18:3 $\Delta^9,12,15$) are essential fatty acids found
in many seed oils. The levels of these fatty-acids
have been manipulated in oil seed crops through
breeding and biotechnology (Ohlrogge, et al. 1991
Biochim. Biophys. Acta 1082:1-26; Topfer et al. 1995
15 *Science* 268:681-686). Additionally, the production of
novel fatty acids in seed oils can be of considerable
use in both human health and industrial applications.

Consumption of plant oils rich in γ -
linolenic acid (GLA) (18:3 $\Delta^6,9,12$) is thought to
20 alleviate hypercholesterolemia and other related
clinical disorders which correlate with susceptibility
to coronary heart disease (Brenner R.R. 1976 *Adv. Exp.*
Med. Biol. 83:85-101). The therapeutic benefits of
dietary GLA may result from its role as a precursor to
25 prostaglandin synthesis (Weete, J.D. 1980 in *Lipid*
Biochemistry of Fungi and Other Organisms, eds. Plenum
Press, New York, pp. 59-62). Linoleic acid (18:2) (LA)
is transformed into gamma linolenic acid (18:3) (GLA)
30 by the enzyme Δ^6 -desaturase.

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1 Few seed oils contain GLA despite high
contents of the precursor linoleic acid. This is due
to the absence of Δ^6 -desaturase activity in most
plants. For example, only borage (*Borago*
5 *officinalis*), evening primrose (*Oenothera biennis*),
and currants (*Ribes nigrum*) produce appreciable
amounts of linolenic acid. Of these three species,
only *Oenothera* and borage are cultivated as a
commercial source for GLA. It would be beneficial if
agronomic seed oils could be engineered to produce GLA
10 in significant quantities by introducing a
heterologous Δ^6 -desaturase gene. It would also be
beneficial if other expression products associated
with fatty acid synthesis and lipid metabolism could
be produced in plants at high enough levels so that
15 commercial production of a particular expression
product becomes feasible.

As disclosed in U.S. Patent No. 5,552,306, a
cyanobacterial Δ^6 -desaturase gene has been recently
isolated. Expression of this cyanobacterial gene in
20 transgenic tobacco resulted in significant but low
level GLA accumulation. (Reddy et al. 1996 *Nature*
Biotech. 14:639-642). Applicant's copending U.S.
Application Serial No. 08,366,779, discloses a Δ^6 -
desaturase gene isolated from the plant *Borago*
25 *officinalis* and its expression in tobacco under the
control of the CaMV 35S promoter. Such expression
resulted in significant but low level GLA and
octadecatetraenoic acid (ODTA or OTA) accumulation in
seeds. Thus, a need exists for a promoter which
30 functions in plants and which consistently directs

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1 high level expression of lipid metabolism genes in
transgenic plant seeds.

Sunflower embryos accumulate two major
classes of storage proteins. These are the 11 S
globulins, soluble in 1 M NaCl, and 2 S albumins,
5 soluble in water (Youle et al. 1981 *Am J. Bot* 68:44-
48). The synthesis, processing and accumulation of 2
S albumin seed proteins have been studied intensively
in *Brassica napus* (Crouch et al., 1983 *J. Mol. Appl.*
Genet. 2:273-284; Ericson et al., 1986 *J. Biol. Chem.*
10 261:14576-14581), pea (Higgins et al., 1986 *Plant Mol.*
Biol. 8:37-45), radish (Laroche-Raynal et al., 1986
Eur. J. Biochem. 157:321-327), castor bean (Lord J.M.,
1985 *Eur. J. Biochem* 146:403-409) and Brazil nut (Sun
et al., 1987 *Eur. J. Biochem* 162:477-483). A major
15 conclusion of these studies is that the characteristic
low molecular weight, disulfide-linked albumin
polypeptides found in mature seeds result from the
extensive processing of larger precursors synthesized
during embryogenesis. Two additional characteristics
20 that define the 2 S albumin seed storage proteins are
high amide content and high frequency of cysteine
residues (Youle et al., 1981).

In sunflower, the 2 S albumins represent
more than 50% of the protein present in seeds (Youle
25 et al., 1981) and consist of two or three closely
related polypeptides with molecular weights of
approximately 19 kDa (Cohen, E.A., 1986 "Analysis of
sunflower 2S seed storage protein genes" MS thesis,
Texas A&M University; Allen et al. 1987 *Plant Mol Biol*
30 5:165-173). The sunflower albumin is apparently

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maintained in a compact structure by intramolecular
1 disulfide bonds resulting in a rapidly migrating
species with an apparent molecular weight of 14 kDa
when analyzed by SDS-polyacrylamide gel
electrophoresis (SDS-PAGE) under non-reducing
5 conditions. When reduced, this species migrates as a
19 kDa polypeptide (Cohen, E.A., 1986). In contrast,
most other 2 S proteins are composed of large and
small subunit polypeptides, derived from a single
precursor, and linked by intermolecular disulfide bonds
10 (Crouch et al. 1983 *J. Mol. Appl. Genet.* 2:273-284;
Ericson et al. 1986 *J. Biol. Chem.* 261:14576-14581;
Sun et al. 1987, *Eur. J. Bioch.* 162:477-483.)

Albumin polypeptides can be detected in
sunflower embryos by 5 days post-fertilization (DPF),
15 2 days before helianthinins are detectable, and
continue to accumulate through seed maturation.
Sunflower albumin mRNAs, also first detected at 5 DPF,
accumulate rapidly in sunflower embryos reaching
maximum prevalence between 12 and 15 DPF. After this
20 time albumin transcripts decrease in prevalence with
kinetics similar to that observed for helianthinin
mRNA (Allen et al. 1987). Functional sunflower
albumin mRNAs are undetectable in dry seeds,
germinated seedlings or leaves (Cohen 1986).

25 A number of albumin cDNAs and genomic clones
have been isolated from different plant species
including sunflower (Allen et al. 1987 *Mol-Gen Genet.*
210:211-218) and pea (Higgins et al. 1986 *J. Biol.*
Chem 261:11124-11130). As in other classes of seed
30 proteins such as *Brassica napus* (Crouch et al., 1983;

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Ericson et al., 1986), 2 S albumin seed proteins are
1 encoded by small gene families.

The present invention provides 5' regulatory
sequences from a sunflower albumin gene which direct
high level expression of lipid metabolism genes in
5 transgenic plants. In accordance with the present
invention, chimeric constructs comprising a sunflower
albumin 5' regulatory region operably linked to coding
sequence for a lipid metabolism gene such as a $\Delta 6$ -
desaturase gene are provided. Transgenic plants
10 comprising the subject chimeric constructs accumulate
GLA to approximately 10% of C18 fatty acids. This is
within the range of accumulation of GLA for *Oenothera*
biennis, a primary commercial source for GLA.

15 SUMMARY OF THE INVENTION

The present invention is directed to 5'
regulatory regions of a sunflower albumin gene. The
5' regulatory regions, when operably linked to either
the coding sequence of a heterologous gene or sequence
20 complementary to a native plant gene, direct
expression of the heterologous gene or complementary
sequence in a plant seed.

The present invention thus provides
expression cassettes and expression vectors comprising
25 an albumin 5' regulatory region operably linked to a
heterologous gene or a sequence complementary to a
native plant gene.

Plant transformation vectors comprising the
expression cassettes and expression vectors are also
30 provided as are plant cells transformed by these

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1 vectors, and plants and their progeny containing the
vectors.

In one embodiment of the invention, the heterologous gene or complementary sequence is a fatty acid synthesis gene or a lipid metabolism gene.

5 In another aspect of the present invention, a method is provided for producing a plant with increased levels of a product of a fatty acid synthesis or lipid metabolism gene.

10 In particular, there is provided a method for producing a plant with increased levels of a fatty acid synthesis or lipid metabolism gene by transforming a plant with the subject expression cassettes and expression vectors which comprise an albumin 5' regulatory region and a coding sequence for
15 a fatty acid synthesis or lipid metabolism gene.

In another aspect of the present invention, there is provided a method for cosuppressing a native fatty acid synthesis or lipid metabolism gene by transforming a plant with the subject expression
20 cassettes and expression vectors which comprise an albumin 5' regulatory region and a coding sequence for a fatty acid synthesis or lipid metabolism gene.

A further aspect of this invention provides a method of decreasing production of a native plant
25 gene such as a fatty acid synthesis gene or a lipid metabolism gene by transforming a plant with an expression vector comprising an albumin 5' regulatory region operably linked to a nucleic acid sequence complementary to a native plant gene.

30

35

Also provided are methods of modulating the
1 levels of a heterologous gene or native plant gene
such as a fatty acid synthesis or lipid metabolism
gene by transforming a plant with the subject
expression cassettes and expression vectors.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts the nucleotide and
corresponding amino acid sequence of the borage $\Delta 6$ -
desaturase gene (SEQ ID NO:1). The cytochrome b5
10 heme-binding motif is boxed and the putative metal
binding, histidine rich motifs (HRMs) are underlined.
The motifs recognized by the primers (PCR analysis)
are underlined with dotted lines, i.e. tgg aaa tgg aac
cat aa; and gag cat cat ttg ttt cc.

15

Fig. 2 is a dendrogram showing similarity of
the borage $\Delta 6$ -desaturase to other membrane-bound
desaturases. The amino acid sequence of the borage $\Delta 6$ -
desaturase was compared to other known desaturases
using Gene Works (IntelliGenetics). Numerical values
20 correlate to relative phylogenetic distances between
subgroups compared.

Fig. 3A provides a gas liquid chromatography
profile of the fatty acid methyl esters (FAMES)
derived from leaf tissue of a wild type tobacco
25 'Xanthi'.

Fig. 3B provides a gas liquid chromatography
profile of the FAMES derived from leaf tissue of a
tobacco plant transformed with the borage $\Delta 6$ -
desaturase cDNA under transcriptional control of the
30 CaMV 35S promoter (pAN2). Peaks corresponding to

1 methyl linoleate (18:2), methyl γ -linolenate (18:3 γ),
2 methyl α -linolenate (18:3 α), and methyl
3 octadecatetraenoate (18:4) are indicated.

4 Fig. 4 is the nucleotide sequence of the
5 HaG5 regulatory region. The transcriptional start
6 site (+1) is indicated by a bold T. The underlined
7 Bam HI restriction site was introduced by PCR.

8 Fig. 5 is a scheme depicting construction of
9 the sunflower albumin HaG5 regulatory region/ $\Delta 6$ -
10 desaturase gene expression vector.

11 Fig. 6A is an RNA gel blot analysis carried
12 out on 5 μ g samples of RNA isolated from borage leaf,
13 root, and 12 dpp embryo tissue, using labeled borage
14 $\Delta 6$ -desaturase cDNA as a hybridization probe.

15 Fig. 6B depicts a graph corresponding to the
16 Northern analysis results for the experiment shown in
17 Fig. 6A.

18 Fig. 7 is a PCR analysis showing the
19 presence of the borage delta 6-desaturase gene in
20 transformed plants of oilseed rape. Lanes 1,3 and 4
21 were loaded with PCR reactions performed with DNA from
22 plants transformed with the borage delta 6-desaturase
23 gene linked to the oleosin 5' regulatory region; lane
24 2: DNA from plant transformed with the borage delta
25 6-desaturase gene linked to the albumin 5' regulatory
26 region; lanes 5 and 6: DNA from non-transformed
27 plants; lane 7: molecular weight marker (1 kb ladder,
28 Gibco BRL); lane 8: PCR without added template DNA;
29 lane 9: control with DNA from *Agrobacterium*
30 *tumefaciens* EHA 105 containing the plasmid pAN3 the

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1 borage delta 6-desaturase gene linked to the oleosin
5' regulatory region.

DETAILED DESCRIPTION OF THE INVENTION

5 The present invention provides isolated
nucleic acids encoding 5' regulatory regions from a
sunflower albumin gene. In accordance with the
present invention, the subject 5' regulatory regions,
when operably linked to either a coding sequence of a
heterologous gene or sequence complementary to a
10 native plant gene, direct expression of the coding
sequence or the complementary sequence in a plant
seed. The albumin 5' regulatory regions of the
present invention are useful in the construction of an
expression cassette which comprises in the 5' to 3'
15 direction, a subject albumin 5' regulatory region, a
heterologous gene or sequence complementary to a
native plant gene under control of the regulatory
region and a 3' termination sequence. Such an
expression cassette can be incorporated into a variety
20 of autonomously replicating vectors in order to
construct an expression vector.

In accordance with the present invention, it
has been surprisingly found that plants transformed
with a subject expression vector accumulate GLA to
25 approximately 10% of C18 fatty acids. Such an
accumulation is within the range of accumulation of
GLA for *Oenothera biennis*, a primary commercial source
for GLA.

As used herein, the term "cassette" refers
30 to a nucleotide sequence capable of expressing a

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1 particular gene if said gene is inserted so as to be
operably linked to one or more regulatory regions
present in the nucleotide sequence. Thus, for
example, the expression cassette may comprise a
5 heterologous coding sequence which is desired to be
expressed in a plant seed. The expression cassettes
and expression vectors of the present invention are
therefore useful for directing seed-specific
expression of any number of heterologous genes. The
term "seed-specific expression" as used herein, refers
10 to expression in various portions of a plant seed such
as the endosperm and embryo.

An isolated nucleic acid encoding a 5'
regulatory region from a sunflower albumin gene can be
provided as follows. Albumin recombinant genomic
15 clones are isolated by screening a sunflower genomic
DNA library with a cDNA (or a portion thereof)
representing albumin mRNA. A number of different
albumin cDNAs have been isolated. The methods used to
isolate such cDNAs as well as the nucleotide and
20 corresponding amino acid sequences have been
published. Higgins et al., 1986 *J. Biol. Chem.* 261:
11124-11130; Allen et al., 1987 in *Molecular
Approaches to Developmental Biology*, Alan R. Liss,
Inc., pp. 415-424.

25 Methods considered useful in obtaining
albumin genomic recombinant DNA are provided in
Sambrook et al. 1989, in *Molecular Cloning: A
Laboratory Manual*, Cold Spring Harbor, NY, for
example, or any of the myriad of laboratory manuals on
30 recombinant DNA technology that are widely available.

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1 To determine nucleotide sequences, a multitude of
techniques are available and known to the ordinarily
skilled artisan. For example, restriction fragments
containing an albumin regulatory region can be
subcloned into the polylinker site of a sequencing
5 vector such as pBluescript (Stratagene). These
pBluescript subclones can then be sequenced by the
double-stranded dideoxy method (Chen and Seeburg,
1985, *DNA* 4:165).

10 In a preferred embodiment, the sunflower
albumin regulatory region comprises nucleotides 860 to
+29 of Fig. 4 (nucleotides 1-895 of SEQ ID NO:2).
Modifications to the albumin regulatory region as set
forth in SEQ ID NO:2 which maintain the characteristic
property of directing seed-specific expression, are
15 within the scope of the present invention. Such
modifications include insertions, deletions and
substitutions of one or more nucleotides.

The 5' regulatory region of the present
invention can be derived from restriction endonuclease
20 or exonuclease digestion of an albumin genomic clone.
Thus, for example, the known nucleotide or amino acid
sequence of the coding region of an isolated albumin
gene is aligned to the nucleic acid or deduced amino
acid sequence of an isolated albumin genomic clone and
25 5' flanking sequence (i.e., sequence upstream from the
translational start codon of the coding region) of the
isolated albumin genomic clone located.

The albumin 5' regulatory region as set
forth in SEQ ID NO:2 (nucleotides -860 to +29 of Fig.
30 4) may be generated from a genomic clone having either

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or both excess 5' flanking sequence or coding sequence
1 by exonuclease III-mediated deletion. This is
accomplished by digesting appropriately prepared DNA
with exonuclease III (exoIII) and removing aliquots at
increasing intervals of time during the digestion.
5 The resulting successively smaller fragments of DNA
may be sequenced to determine the exact endpoint of
the deletions. There are several commercially
available systems which use exonuclease III (exoIII)
to create such a deletion series, e.g. Promega
10 Biotech, "Erase-A-Base" system. Alternatively, PCR
primers can be defined to allow direct amplification
of the subject 5' regulatory regions.

Using the same methodologies, the
ordinarily skilled artisan can generate one or more
15 deletion fragments of nucleotides 1-895 as set forth
in SEQ ID NO:2. Any and all deletion fragments which
comprise a contiguous portion of nucleotides set forth
in SEQ ID NO:2 and which retain the capacity to direct
seed-specific expression are contemplated by the
20 present invention.

The identification of albumin 5' regulatory
sequences which direct seed-specific expression
comprising nucleotides 1-895 of SEQ ID NO:2 and
modifications or deletion fragments thereof, can be
25 accomplished by transcriptional fusions of specific
sequences with the coding sequences of a heterologous
gene, transfer of the chimeric gene into an
appropriate host, and detection of the expression of
the heterologous gene. The assay used to detect
30 expression depends upon the nature of the heterologous

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sequence. For example, reporter genes, exemplified by
1 chloramphenicol acetyl transferase and β -glucuronidase
(GUS), are commonly used to assess transcriptional and
translational competence of chimeric constructions.
Standard assays are available to sensitively detect
5 the reporter enzyme in a transgenic organism. The β -
glucuronidase (GUS) gene is useful as a reporter of
promoter activity in transgenic plants because of the
high stability of the enzyme in plant cells, the lack
of intrinsic β -glucuronidase activity in higher plants
10 and availability of a quantitative fluorimetric assay
and a histochemical localization technique.
Jerfferson et al. (1987 *EMBO J* 6:3901) have
established standard procedures for biochemical and
histochemical detection of GUS activity in plant
15 tissues. Biochemical assays are performed by mixing
plant tissue lysates with 4-methylumbelliferyl- β -D-
glucuronide, a fluorimetric substrate for GUS,
incubating one hour at 37°C, and then measuring the
fluorescence of the resulting 4-methyl-umbelliferone.
20 Histochemical localization for GUS activity is
determined by incubating plant tissue samples in 5-
bromo-4-chloro-3-indolyl-glucuronide (X-Gluc) for
about 18 hours at 37°C and observing the staining
pattern of X-Gluc. The construction of such chimeric
25 genes allows definition of specific regulatory
sequences and demonstrates that these sequences can
direct expression of heterologous genes in a seed-
specific manner.

Another aspect of the invention is directed
30 to a chimeric plant gene comprising a 5' regulatory

35

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1 region from an albumin gene which directs seed
specific expression operably linked to the coding
sequence of a heterologous gene such that the
regulatory element is capable of controlling
expression of the product encoded by the heterologous
5 gene. The heterologous gene can be any gene other
than albumin. If necessary, additional regulatory
elements or parts of these elements sufficient to
cause expression resulting in production of an
effective amount of the polypeptide encoded by the
10 heterologous gene are included in the chimeric
constructs.

Accordingly, the present invention provides
chimeric genes comprising sequences of the albumin 5'
regulatory region that confer seed-specific expression
15 which are operably linked to a sequence encoding a
heterologous gene such as a lipid metabolism enzyme.
Examples of lipid metabolism and fatty acid synthesis
genes useful for practicing the present invention
include lipid desaturases such as $\Delta 6$ -desaturases, $\Delta 12$ -
20 desaturases, $\Delta 15$ -desaturases and other related
desaturases such as stearyl-ACP desaturases, acyl
carrier proteins (ACPs), thioesterases, acetyl
transacylases, acetyl-coA carboxylases, ketoacyl-
synthases, malonyl transacylases, and elongases. Such
25 lipid metabolism and fatty acid synthesis genes have
been isolated and characterized from a number of
different bacteria and plant species. Their
nucleotide coding sequences as well as methods of
isolating such coding sequences are disclosed in the
30

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1 published literature and are widely available to those
of skill in the art.

In particular, the $\Delta 6$ -desaturase genes disclosed in U.S. Patent No. 5,552,306 and applicants' copending U.S. Application Serial No. 5 08/366,779 filed December 30, 1994 and incorporated herein by reference, are contemplated as lipid metabolism genes particularly useful in the practice of the present invention.

The chimeric genes of the present invention 10 are constructed by ligating a 5' regulatory region of an albumin genomic DNA to the coding sequence of a heterologous gene. The juxtaposition of these sequences can be accomplished in a variety of ways. In a preferred embodiment the order of the sequences, 15 from 5' to 3', is an albumin 5' regulatory region (including a promoter), a coding sequence, and a termination sequence which includes a polyadenylation site.

Standard techniques for construction of such 20 chimeric genes are well known to those of ordinary skill in the art and can be found in references such as Sambrook et al. (1989). A variety of strategies are available for ligating fragments of DNA, the choice of which depends on the nature of the termini of the DNA 25 fragments. One of ordinary skill in the art recognizes that in order for the heterologous gene to be expressed, the construction requires promoter elements and signals for efficient polyadenylation of the transcript. Accordingly, the albumin 5' 30 regulatory region that contains the consensus promoter

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sequence known as the TATA box can be ligated directly
1 to a promoterless heterologous coding sequence.

The restriction or deletion fragments that
contain the albumin TATA box are ligated in a forward
orientation to a promoterless heterologous gene such
5 as the coding sequence of β -glucuronidase (GUS). The
skilled artisan will recognize that the subject
albumin 5' regulatory regions can be provided by other
means, for example chemical or enzymatic synthesis.
The 3' end of a heterologous coding sequence is
10 optionally ligated to a termination sequence
comprising a polyadenylation site, exemplified by, but
not limited to, the nopaline synthase polyadenylation
site, or the octopine T-DNA gene 7 polyadenylation
site. Alternatively, the polyadenylation site can be
15 provided by the heterologous gene.

The present invention also provides methods
of increasing levels of heterologous genes in plant
seeds. In accordance with such methods, the subject
expression cassettes and expression vectors are
20 introduced into a plant in order to effect expression
of a heterologous gene. For example, a method of
producing a plant with increased levels of a product
of a fatty acid synthase or lipid metabolism gene is
provided by transforming a plant cell with an
25 expression vector comprising an albumin 5' regulatory
region operably linked to a fatty acid synthesis or
lipid metabolism gene and regenerating a plant with
increased levels of the product of said fatty acid
synthesis or lipid metabolism gene.

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Another aspect of the present invention
1 provides methods of reducing levels of a product of a
gene which is native to a plant which comprises
transforming a plant cell with an expression vector
comprising a subject albumin regulatory region
5 operably linked to a nucleic acid sequence which is
complementary to the native plant gene. In this
manner, levels of endogenous product of the native
plant gene are reduced through the mechanism known as
antisense regulation. Thus, for example, levels of a
10 product of a fatty acid synthesis gene or lipid
metabolism gene are reduced by transforming a plant
with an expression vector comprising a subject albumin
5' regulatory region operably linked to a nucleic acid
sequence which is complementary to a nucleic acid
15 sequence coding for a fatty acid synthesis or lipid
metabolism gene.

The present invention also provides a method
of cosuppressing a gene which is native to a plant
which comprises transforming a plant cell with an
20 expression vector comprising a subject albumin
regulatory region operably linked to a nucleic acid
sequence coding for the native plant gene. In this
manner, levels of endogenous product of the native
plant gene are reduced through the mechanism known as
25 cosuppression. Thus, for example, levels of a product
of a fatty acid synthesis gene or lipid metabolism
gene are reduced by transforming a plant with an
expression vector comprising a subject albumin 5'
regulatory region operably linked to a nucleic acid
30 sequence coding for a fatty acid synthesis or lipid

1 metabolism gene native to the plant. Although the
exact mechanism of cosuppression is not completely
understood, one skilled in the art is familiar with
published works reporting the experimental conditions
and results associated with cosuppression (Napoli et
5 al. 1990 *The Plant Cell* 2:270-289; Van der Krol 1990
The Plant Cell 2:291-299.

To provide regulated expression of the
heterologous or native genes, plants are transformed
with the chimeric gene constructions of the invention.
10 Methods of gene transfer are well known in the art.
The chimeric genes can be introduced into plants by
leaf disk transformation-regeneration procedure as
described by Horsch et al. (1985) *Science* 227:1229.
Other methods of transformation such as protoplast
15 culture (Horsch et al. 1984 *Science* 223:496, DeBlock
et al. 1984 *EMBO J.* 2:2143, Barton et al. 1983, *Cell*
32:1033) can also be used and are within the scope of
this invention. In a preferred embodiment, plants are
transformed with *Agrobacterium*-derived vectors such as
20 those described in Klett et al. (1987) *Annu. Rev.*
Plant Physiol. 38:467. Other well-known methods are
available to insert the chimeric genes of the present
invention into plant cells. Such alternative methods
include biolistic approaches (Klein et al. 1987 *Nature*
25 327:70), electroporation, chemically-induced DNA
uptake, and use of viruses or pollen as vectors.

When necessary for the transformation
method, the chimeric genes of the present invention
can be inserted into a plant transformation vector,
30 e.g. the binary vector described by Bevan, M. 1984

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1 Nucl. Acids Res. 12:8711-8721. Plant transformation
vectors can be derived by modifying the natural gene
transfer system of *Agrobacterium tumefaciens*. The
natural system comprises large Ti (tumor-inducing)-
5 plasmids containing a large segment, known as T-DNA,
which is transferred to transformed plants. Another
segment of the Ti plasmid, the *vir* region, is
responsible for T-DNA transfer. The T-DNA region is
bordered by terminal repeats. In the modified binary
10 vectors, the tumor inducing genes have been deleted
and the functions of the *vir* region are utilized to
transfer foreign DNA bordered by the T-DNA border
sequences. The T-region also contains a selectable
marker for antibiotic resistance, and a multiple
15 cloning site for inserting sequences for transfer.
Such engineered strains are known as "disarmed" *A.*
tumefaciens strains, and allow the efficient transfer
of sequences bordered by the T-region into the nuclear
genome of plants.

20 Surface-sterilized leaf disks or other
susceptible tissues are inoculated with the "disarmed"
foreign DNA-containing *A. tumefaciens*, cultured for a
number of days, and then transferred to antibiotic-
containing medium. Transformed shoots are then
selected after rooting in medium containing the
25 appropriate antibiotic, and transferred to soil.
Transgenic plants are pollinated and seeds from these
plants are collected and grown on antibiotic medium.

30 Expression of a heterologous or reporter
gene in developing seeds, young seedlings and mature
plants can be monitored by immunological,

1 histochemical or activity assays. As discussed herein,
the choice of an assay for expression of the chimeric
gene depends upon the nature of the heterologous
coding region. For example, Northern analysis can be
used to assess transcription if appropriate nucleotide
5 probes are available. If antibodies to the
polypeptide encoded by the heterologous gene are
available, Western analysis and immunohistochemical
localization can be used to assess the production and
localization of the polypeptide. Depending upon the
10 heterologous gene, appropriate biochemical assays can
be used. For example, acetyltransferases are detected
by measuring acetylation of a standard substrate. The
expression of a lipid desaturase gene can be assayed
by analysis of fatty acid methyl esters (FAMES).

15 Another aspect of the present invention
provides transgenic plants or progeny of these plants
containing the chimeric genes of the invention. Both
monocotyledonous and dicotyledonous plants are
contemplated. Plant cells are transformed with the
20 chimeric genes by any of the plant transformation
methods described above. The transformed plant cell,
usually in the form of a callus culture, leaf disk or
whole plant (via the vacuum infiltration method of
Bechtold et al. 1993 *C.R. Acad. Sci. Paris*, 316:1194-
25 1199) is regenerated into a complete transgenic plant
by methods well-known to one of ordinary skill in the
art (e.g. Horsh et al. 1985 *Science* 227:1129). In a
preferred embodiment, the transgenic plant is
sunflower, cotton, oil seed rape, maize, tobacco,
30 *Arabidopsis*, peanut or soybean. Since progeny of

- 22 -

transformed plants inherit the chimeric genes, seeds
1 or cuttings from transformed plants are used to
maintain the transgenic line.

The following examples further illustrate
the invention.

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EXAMPLE 1

1 **Isolation of Membrane-Bound Polysomal
RNA and Construction of Borage cDNA Library**

5 Membrane-bound polysomes were isolated from
borage seeds 12 days post pollination (12 DPP) using
the protocol established for peas by Larkins and
Davies (1975 *Plant Phys.* 55: 749-756). RNA was
10 extracted from the polysomes as described by Mechler
(1987 *Methods in Enzymology* 152: 241-248, Academic
Press). Poly-A⁺ RNA was isolated from the membrane
bound polysomal RNA using Oligotex-dT[™] beads (Qiagen).

 Corresponding cDNA was made using
Stratagene's ZAP cDNA synthesis kit. The cDNA library
15 was constructed in the lambda ZAP II vector
(Stratagene) using the lambda ZAP II kit. The primary
library was packaged with Gigapack II Gold packaging
extract (Stratagene).

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EXAMPLE 21 Isolation of a Δ -6 Desaturase cDNA from Borage5 Hybridization protocol

5 The amplified borage cDNA library was plated at low density (500 pfu on 150 mm petri dishes). Highly prevalent seed storage protein cDNAs were reduced (subtracted from the total cDNAs) by screening with the corresponding cDNAs.

10 Hybridization probes for screening the borage cDNA library were generated by using random primed DNA synthesis as described by Ausubel et al (1994 Current Protocols in Molecular Biology, Wiley Interscience, N.Y.) and corresponded to previously
15 identified abundantly expressed seed storage protein cDNAs. Unincorporated nucleotides were removed by use of a G-50 spin column (Boehringer Mannheim). Probe was denatured for hybridization by boiling in a water bath for 5 minutes, then quickly cooled on ice.

20 Nitrocellulose filters carrying fixed recombinant bacteriophage were prehybridized at 60°C for 2-4 hours in hybridization solution [4X SET (600 mM NaCl, 80 mM Tris-HCl, 4 mM Na₂EDTA; pH 7.8), 5X Denhardt's reagent (0.1% bovine serum albumin, 0.1% Ficoll, and 0.1%
25 polyvinylpyrrolidone), 100 µg/ml denatured salmon sperm DNA, 50 µg/ml polyadenine and 10 µg/ml polycytidine]. This was replaced with fresh hybridization solution to which denatured radioactive probe (2 ng/ml hybridization solution) was added. The filters were
30 incubated at 60°C with agitation overnight. Filters

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were washed sequentially in 4X, 2X, and 1X SET (150 mM
1 NaCl, 20 mM Tris-HCl, 1 mM Na₂EDTA; pH7.8) for 15
minutes each at 60°C. Filters were air dried and then
exposed to X-ray film for 24 hours with intensifying
screens at -80°C.

5 Non-hybridizing plaques were excised using
Stratagene's excision protocol and reagents.
Resulting bacterial colonies were used to inoculate
liquid cultures and were either sequenced manually or
by an ABI automated sequencer.

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Random Sequencing of cDNAs from a Borage Seed 12 (DPP)
Membrane-Bound Polysomal Library

Each cDNA corresponding to a non-
hybridizing plaque was sequenced once and a sequence
tag generated from 200-300 base pairs. All sequencing
15 was performed by cycle sequencing (Epicentre). Over
300 expressed sequence tags (ESTs) were generated.
Each sequence tag was compared to GenBank database
using the BLAST algorithm (Altschul et al. 1990 *J.*
20 *Mol. Biol.* 215:403-410). A number of lipid metabolism
genes, including the Δ6-desaturase were identified.

Database searches with the cDNA clone
designated mbp-65 using BLASTX with the GenBank
database resulted in a significant match to the
previously isolated *Synechocystis* Δ6-desaturase. It
25 was determined however, that mbp-65 was not a full
length cDNA. A full length cDNA was isolated using
mbp-65 to screen the borage membrane-bound polysomal
library. The resultant clone was designated pAN1 and
the cDNA insert of pAN1 was sequenced by the cycle
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sequencing method. The amino acid sequence deduced
1 from the open reading frame (Fig. 1, SEQ ID NO:1) was
compared to other known desaturases using Geneworks
(IntelligGenetics) protein alignment program. This
alignment indicated that the cDNA insert of pAN1 was
5 the borage Δ^6 -desaturase gene.

The resulting dendrogram (Figure 2) shows
that Δ^{15} -desaturases and Δ^{12} -desaturases comprise two
groups. The newly isolated borage sequence and the
previously isolated *Synechocystis* Δ^6 -desaturase (U.S.
10 Patent No. 5,552,306) formed a third distinct group.
A comparison of amino acid motifs common to
desaturases and thought to be involved catalytically
in metal binding illustrates the overall similarity of
the protein encoded by the borage gene to desaturases
15 in general and the *Synechocystis* Δ^6 -desaturase in
particular (Table 1). At the same time, comparison of
the motifs in Table 1 indicates definite differences
between this protein and other plant desaturases.
Furthermore, the borage sequence is also distinguished
20 from known plant membrane associated fatty acid
desaturases by the presence of a heme binding motif
conserved in cytochrome b₅ proteins (Schmidt et al.
1994 *Plant Mol. Biol.* 26:631-642) (Figure 1). Thus,
while these results clearly suggested that the
25 isolated cDNA was a borage Δ^6 -desaturase gene, further
confirmation was necessary. To confirm the identity
of the borage Δ^6 -desaturase cDNA, the cDNA insert from
pAN1 was cloned into an expression cassette for stable
expression. The vector pBI121 (Jefferson et al. 1987
30 *EMBO J.* 6:3901-3907) was prepared for ligation by

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digestion with BamHI and EcoICR I (an isoschizomer of
1 SacI which leaves blunt ends; available from Promega)
which excises the GUS coding region leaving the 35S
promoter and NOS terminator intact. The borage Δ^6 -
desaturase cDNA was excised from the recombinant
5 plasmid (pAN1) by digestion with BamHI and XhoI. The
XhoI end was made blunt by performing a fill-in
reaction catalyzed by the Klenow fragment of DNA
polymerase I. This fragment was then cloned into the
BamHI/EcoICR I sites of pBI121.1, resulting in the
10 plasmid pAN2.

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TABLE 1
COMPARISON OF COMMON AMINO ACID MOTIFS IN MEMBRANE-BOUND DESATURASES

Desaturase	Lipid Box	Metal Box 1	Metal Box 2
Borage Δ^6	WIGHDAGH (SEQ. ID. NO:3)	HNAHH (SEQ. ID. NO:9)	FQIEHH (SEQ. ID. NO:17)
Synechocystis Δ^6	NVGHDANH (SEQ. ID. NO:4)	HNYLHH (SEQ. ID. NO:10)	HQVTHH (SEQ. ID. NO:18)
Arab. chloroplast Δ^{15}	VLGHDCGH (SEQ. ID. NO:5)	HRTHH (SEQ. ID. NO:11)	HVTIHH (SEQ. ID. NO:19)
Rice Δ^{15}	VLGHDCGH (SEQ. ID. NO:5)	HRTHH (SEQ. ID. NO:11)	HVTIHH (SEQ. ID. NO:19)
Glycine chloroplast Δ^{15}	VLGHDCGH (SEQ. ID. NO:5)	HRTHH (SEQ. ID. NO:11)	HVTIHH (SEQ. ID. NO:19)
Arab. fad3 (Δ^{15})	VLGHDCGH (SEQ. ID. NO:5)	HRTHH (SEQ. ID. NO:11)	HVTIHH (SEQ. ID. NO:19)
Brassica fad 3 (Δ^{15})	VLGHDCGH (SEQ. ID. NO:5)	HRTHH (SEQ. ID. NO:11)	HVTIHH (SEQ. ID. NO:19)
Borage Δ^{11} (P1-81)*	VIAHECGH (SEQ. ID. NO:6)	HRRHH (SEQ. ID. NO:12)	HVAHH (SEQ. ID. NO:20)
Arab. fad2 (Δ^{11})	VIAHECGH (SEQ. ID. NO:6)	HRRHH (SEQ. ID. NO:12)	HVAHH (SEQ. ID. NO:20)
Arab. chloroplast Δ^{11}	VIGHDCAH (SEQ. ID. NO:7)	HDRHH (SEQ. ID. NO:13)	HIPHH (SEQ. ID. NO:21)
Glycine plastid Δ^{11}	VIGHDCAH (SEQ. ID. NO:7)	HDRHH (SEQ. ID. NO:13)	HIPHH (SEQ. ID. NO:21)
Spinach plastidial n-6	VIGHDCAH (SEQ. ID. NO:7)	HDQHH (SEQ. ID. NO:14)	HIPHH (SEQ. ID. NO:21)
Synechocystis Δ^{11}	VVGHDCGH (SEQ. ID. NO:8)	HDHHH (SEQ. ID. NO:15)	HIPHH (SEQ. ID. NO:21)
Anabaena Δ^{11}	VLGHDCGH (SEQ. ID. NO:5)	HNHHH (SEQ. ID. NO:16)	HVPHH (SEQ. ID. NO:22)

*P1-81 is a full length cDNA which was identified by EST analysis and shows high similarity to the Arabidopsis Δ^{12} desaturase (fad2)

EXAMPLE 3

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Production of Transgenic
Plants and Preparation and
Analysis of Fatty Acid Methyl Esters (FAMES)

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The expression plasmid, pAN2 was used to transform tobacco (*Nicotiana tabacum* cv. *xanthi*) via *Agrobacterium tumefaciens* according to standard procedures (Horsch, et al. 1985 *Science* 227:1229-1231; Bogue et al. 1990 *Mol. Gen. Genet.* 221:49-57) except that the initial transformants were selected on 100 μ g/ml kanamycin.

Tissue from transgenic plants was frozen in liquid nitrogen and lyophilized overnight. FAMES were prepared as described by Dahmer, et al. (1989) *J. Amer. Oil. Chem. Soc.* 66: 543-548. In some cases, the solvent was evaporated again, and the FAMES were resuspended in ethyl acetate and extracted once with deionized water to remove any water soluble contaminants. FAMES were analyzed using a Tracor-560 gas liquid chromatograph as previously described (Reddy et al. 1996 *Nature Biotech.* 14:639-642).

As shown in Figure. 3, transgenic tobacco leaves containing the borage cDNA produced both GLA and octadecatetraenoic acid (OTA) (18:4 Δ 6,9,12,15). These results thus demonstrate that the isolated cDNA encodes a borage Δ 6-desaturase.

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EXAMPLE 41 Expression of $\Delta 6$ -desaturase in Borage

 The native expression of $\Delta 6$ -desaturase was
examined by Northern Analysis of RNA derived from
5 borage tissues. RNA was isolated from developing
borage embryos following the method of Chang et al.
1993 *Plant Mol. Biol. Rep.* 11:113-116.
RNA was electrophoretically separated on formaldehyde-
agarose gels, blotted to nylon membranes by capillary
10 transfer, and immobilized by baking at 80°C for 30
minutes following standard protocols (Brown T., 1996
in *Current Protocols in Molecular Biology*, eds.
Auselbel, et al. [Greene Publishing and Wiley-
Interscience, New York] pp. 4.9.1-4.9.14.). The
15 filters were preincubated at 42°C in a solution
containing 50% deionized formamide, 5X Denhardt's
reagent, 5X SSPE (900 mM NaCl; 50mM Sodium phosphate,
pH7.7; and 5 mM EDTA), 0.1% SDS, and 200 μ g/ml
denatured salmon sperm DNA. After two hours, the
20 filters were added to a fresh solution of the same
composition with the addition of denatured radioactive
hybridization probe. In this instance, the probes
used were borage legumin cDNA (Fig. 16A), borage
oleosin cDNA (Fig. 16B), and borage $\Delta 6$ -desaturase cDNA
25 (pAN1, Example 2) (Fig. 16C). The borage legumin and
oleosin cDNAs were isolated by EST cloning and
identified by comparison to the GenBank database using
the BLAST algorithm as described in Example 2.
Loading variation was corrected by normalizing to
30 levels of borage EF1 α mRNA. EF1 α mRNA was identified

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1 by correlating to the corresponding cDNA obtained by
the EST analysis described in Example 2. The filters
were hybridized at 42°C for 12-20 hours, then washed
as described above (except that the temperature was
65°C), air dried, and exposed to X-ray film.

5 As depicted in Figs. 15A and 15B, $\Delta 6$ -
desaturase is expressed primarily in borage seed.
Borage seeds reach maturation between 18-20 days post
pollination (dpp). $\Delta 6$ -desaturase mRNA expression
occurs throughout the time points collected (8-20
10 dpp), but appears maximal from 10-16 days post
pollination. This expression profile is similar to
that seen for borage oleosin and 12S seed storage
protein mRNAs (Figs. 16A, 16B, and 16C).

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EXAMPLE 5

1 ISOLATION OF A SUNFLOWER ALBUMIN cDNA

5 The sunflower albumin cDNA (Ha5) was
isolated by differentially screening a sunflower cDNA
library using cDNA probes from leaf and 12 DPF (days
post flowering) embryos. A cDNA of 1011 bp was
obtained (Cohen E.A. "Analysis of sunflower 2S seed
storage protein genes" MS thesis, Texas A&M
10 University, Allen et al., 1987a in *Molecular
Approaches to Developmental Biology*, pp. 415-424.).
Although not full length, the cDNA comprised most of
the coding sequence for the sunflower 2S albumin.
Northern and dot blot analysis indicated that this
15 gene is exclusively expressed in developing sunflower
seeds. Albumin transcripts and protein are first
detected 5 DPF, a full two days earlier than
helianthinin (11S), and reach maximal prevalence
around 12-15 DPF (Allen et al. 1987a).
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EXAMPLE 61 ISOLATION OF A SUNFLOWER ALBUMIN 5' REGULATORY REGION

Genomic clones were isolated by screening a
sunflower genomic DNA library using the Ha5 cDNA as a
5 probe. Four independent genomic clones were shown to
be identical by restriction enzyme digestion.
Therefore, one clone (HaG5) was chosen for more
detailed analysis.

A 2.3kb EcoRI/DraI fragment was sequenced
10 (Allen et al., 1987b *Mol. Gen. Genet.* 210: 211-218).
The HaG5 albumin gene contains two exons. The first
exon (exon 1) is 575 nucleotides in length and the
second exon (exon 2) is 310 nucleotides in length. A
190 nucleotide intron separates the two exons.
15 Nuclease protection experiments showed that the
transcription start site was located 30 nucleotides
upstream of the translational start site. (Allen et
al 1987b, Fig. 2). Southern analysis of genomic DNA
and the fact that only one gene was isolated in an
20 exhaustive screen indicated that HaG5 is a single copy
gene in the sunflower genome.

An 889 bp upstream regulatory region (-860
to +29 of Figure 4; SEQ ID NO:2) was cloned in several
steps from HaG5. A 1.1 kb EcoRI fragment was
25 subcloned in PBluescript™ (Stratagene) yielding
pHaG5RI. PCR was performed on pHaG5RI with primers
that resulted in the albumin 5' regulatory region
being flanked by EcoRI and BamHI sites at the 5' and
3' ends, respectively. The restriction fragment was
30 cloned into the EcoRI/BamHI sites of pBluescript™

yielding pHaG5EB. Individual clones were sequenced to
1 check possible PCR mutations as well as the
orientation of their inserts. The sequence of the
albumin 5' regulatory region is shown in Fig. 4 (SEQ.
ID NO:2). The SalI/BamHI fragment of this construct
5 was excised and cloned into pAN3 (the parental borage
 $\Delta 6$ -desaturase containing plasmid), yielding pAN4. A
map of pAN4 and the intermediate vectors involved in
its construction are shown in Fig. 5. pAN1 is
described in Example 2. pBI101.1 is described in
10 (Jefferson et al. 1987 *EMBO J.* 6:3901-3907).

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EXAMPLE 7

1 **EXPRESSION OF Δ^6 -DESATURASE UNDER CONTROL OF THE**
 SUNFLOWER ALBUMIN 5' REGULATORY REGION

 The albumin 5' regulatory region was used to
5 drive the expression of a borage Δ^6 -desaturase gene in
 Arabidopsis. pAN4 was used to transform *Arabidopsis*
 using the vacuum infiltration method of Bechtold et
 al. 1993 *C.R. Acad. Sci. Paris* 316: 1194-1199. Levels
 of Δ^6 -desaturase activity were monitored by assaying
10 the corresponding fatty acid methyl esters of its
 reaction products, γ -linolenic acid (GLA) and
 octadecatetraenoic acid (OTA) using the methods
 described in Example 3. GLA and OTA levels in
 transgenic seeds ranged up to 10.2% (average of 4.4%)
15 and 3.6% (average of 1.7%), respectively, of the C18
 fatty acids. No GLA or OTA was detected in the leaves
 of these plants. In comparison, 35S promoter/ Δ^6 -
 desaturase transgenic plants produced GLA levels of up
 to 3.1% of C18 fatty acids (average of 1.3%) in leaves
20 and no measurable OTA in seeds. These data are
 summarized in Table 2.

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TABLE 2
EXPRESSION OF THE BORAGE Δ^6 -DESATURASE IN TRANSGENIC PLANTS

PROMOTER	PLANT	SEED			LEAF	
		GLA* RANGE	RANGE	OTA*	GLA RANGE	OTA
Cauliflower mosaic virus 35S	tobacco	1.3	0.7-3.1	n.d	20 8-11	19-22 9.7
Sunflower albumin	Arabidops is	4.4 0.63-	3.1-10.2 3.6	1.7	n.d.	n.d

*mean value expressed as the percent of the C₁₈ fatty acids n.d. not detected

EXAMPLE 8

- 1 Transformation of Oilseed Rape With an Expression
Cassette Which Comprises the Albumin 5' Regulatory
Region Linked to the Borage Delta 6-Desaturase Gene

Oilseed rape, Cv. Westar, was transformed
5 with the strain of *Agrobacterium tumefaciens* EHA105
containing the plasmid pAN4 (i.e. the borage $\Delta 6$ -
desaturase gene under the control of the sunflower
albumin promoter-Example 6).

Terminal internodes of Westar were co-
10 cultivated for 2-3 days with induced *Agrobacterium*
tumefaciens strain EHA105 (Alt-Moerbe et al. 1988 *Mol.*
Gen. Genet. 213:1-8; James et al. 1993 *Plant Cell*
Reports 12:559-563), then transferred onto
regeneration medium (Boulter et al. 1990 *Plant Science*
15 70:91-99; Fry et al. 1987 *Plant Cell Reports* 6:321-
325). The regenerated shoots were transferred to
growth medium (Pelletier et al. 1983 *Mol. Gen. Menet.*
191:244-250), and a polymerase chain reaction (PCR)
test was performed on leaf fragments to assess the
20 presence of the gene.

DNA was isolated from the leaves according
to the protocol of K.M. Haymes et al. (1996) *Plant*
Molecular Biology Reporter, 14(3):280-284, and
resuspended in 100 μ l of water, without RNase
25 treatment. 5 μ l of extract were used for the PCR
reaction, in a final volume of 50 μ l. The reaction was
performed in a Perkin-Elmer 9600 thermocycler, with
the following cycles:

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1 cycle: 95°C, 5 minutes
1 30 cycles: 95°C, 45 sec; 52°C, 45 sec
72°C, 1 minute

1 cycle: 72°C, 5 minutes

5 and the following primers (derived from near the metal box regions, as indicated in Fig. 1, SEQ. NO.:1):

5' TGG AAA TGG AAC CAT AA 3'

5' GGA AAC AAA TGA TGC TC 3'

Amplification of the DNA revealed the expected 549
10 base pair PCR fragment (Figure 7).

The positive shoots were transferred to elongation medium, then to rooting medium (DeBlock et al 1989 *Plant Physiol.* 91:694-701). Shoots with a well-developed root system were transferred to the
15 greenhouse. When plants were well developed, leaves were collected for Southern analysis in order to assess gene copy number.

Genomic DNA was extracted according to the procedure of Bouchez et al. (1996) *Plant Molecular*
20 *Biology Reporter* 14:115-123, digested with the restriction enzymes *Bgl* I and/or *Cla* I, electrophoretically separated on agarose gel (Maniatis et al. 1982, in *Molecular Cloning; a Laboratory*
25 *Spring Harbor/NY*), and prepared for transfer to nylon membranes (Nytran membrane, Schleicher & Schuell) according to the instructions of the manufacturer. DNA was then transferred to membranes overnight by capillary action using 20XSSC (Maniatis et al. 1982).
30 Following transfer, the membranes were crosslinked by

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UV (Stratagene) for 30 seconds and pre-hybridized for
1 1 hour at 65°C in 15 ml of a solution containing
6XSSC, 0.5%SDS and 2.25% w/w dehydrated skim milk in
glass vials in hybridization oven (Appligene). The
membranes were hybridized overnight in the same
5 solution containing a denatured hybridization probe
radiolabelled with ^{32}P to a specific activity of 10^8
cpm/ μg by the random primer method (with the Ready-To-
Go kit obtained from Pharmacia). The probe represents
a PCR fragment of the borage delta 6-desaturase gene
10 (obtained in the conditions and with the primers
detailed above). After hybridization, the filters
were washed at 65°C in 2XSSC, 0.1% SDS for 15 minutes,
and 0.2XSSC, 0.1%SDS for 15 minutes. The membranes
were then wrapped in Saran-Wrap and exposed to Kodak
15 XAR film using an intensifying screen at -70°C in a,
light-proof cassette. Exposure time is generally 3
days.

The results obtained confirm the presence of
the gene. According to the gene construct, the number
20 of bands in each lane of DNA digested by *Bgl* I or *Cla*
I represents the number of delta 6-desaturase genes
present in the genomic DNA of the plant. The
digestion with *Bgl* I and *Cla* I together generates a
fragment of 3058 bp.

25 The term "comprises" or "comprising" is
defined as specifying the presence of the stated features,
integers, steps, or components as referred to in the
claims, but does not preclude the presence or addition
of one or more other features, integers, steps,
30 components, or groups thereof.

-40-

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Rhone Poulenc Agro
Thomas, Terry L.
Nunberg, Andrew N.
Beremand, Phillip D.
- (ii) TITLE OF INVENTION: A SUNFLOWER ALBUMIN 5' REGULATORY REGION
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COMPOSITION
- (iii) NUMBER OF SEQUENCES: 22
- (iv) CORRESPONDENCE ADDRESS:
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- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
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 - (A) NAME: DiGiglio, Frank S.
 - (B) REGISTRATION NUMBER: 31,346
 - (C) REFERENCE/DOCKET NUMBER: 10545
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (516) 742-4343
 - (B) TELEFAX: (516) 743-4366

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1684 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 43..1387

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATATCTGCCT ACCCTCCCAA AGAGAGTAGT CATTTTTCAT CA ATG GCT GCT CAA	54
Met Ala Ala Gln	
1	
ATC AAG AAA TAC ATT ACC TCA GAT GAA CTC AAG AAC CAC GAT AAA CCC	102
Ile Lys Lys Tyr Ile Thr Ser Asp Glu Leu Lys Asn His Asp Lys Pro	
5 10 15 20	
GGA GAT CTA TGG ATC TCG ATT CAA GGG AAA GCC TAT GAT GTT TCG GAT	150
Gly Asp Leu Trp Ile Ser Ile Gln Gly Lys Ala Tyr Asp Val Ser Asp	
25 30 35	
TGG GTG AAA GAC CAT CCA GGT GGC AGC TTT CCC TTG AAG AGT CTT GCT	198
Trp Val Lys Asp His Pro Gly Gly Ser Phe Pro Leu Lys Ser Leu Ala	
40 45 50	
GGT CAA GAG GTA ACT GAT GCA TTT GTT GCA TTC CAT CCT GCC TCT ACA	246
Gly Gln Glu Val Thr Asp Ala Phe Val Ala Phe His Pro Ala Ser Thr	
55 60 65	
TGG AAG AAT CTT GAT AAG TTT TTC ACT GGG TAT TAT CTT AAA GAT TAC	294
Trp Lys Asn Leu Asp Lys Phe Phe Thr Gly Tyr Tyr Leu Lys Asp Tyr	
70 75 80	
TCT GTT TCT GAG GTT TCT AAA GAT TAT AGG AAG CTT GTG TTT GAG TTT	342
Ser Val Ser Glu Val Ser Lys Asp Tyr Arg Lys Leu Val Phe Glu Phe	
85 90 95 100	
TCT AAA ATG GGT TTG TAT GAC AAA AAA GGT CAT ATT ATG TTT GCA ACT	390
Ser Lys Met Gly Leu Tyr Asp Lys Lys Gly His Ile Met Phe Ala Thr	
105 110 115	

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TTG TGC TTT ATA GCA ATG CTG TTT GCT ATG AGT GTT TAT GGG GTT TTG Leu Cys Phe Ile Ala Met Leu Phe Ala Met Ser Val Tyr Gly Val Leu 120 125 130	438
TTT TGT GAG GGT GTT TTG GTA CAT TTG TTT TCT GGG TGT TTG ATG GGG Phe Cys Glu Gly Val Leu Val His Leu Phe Ser Gly Cys Leu Met Gly 135 140 145	486
TTT CTT TGG ATT CAG AGT GGT TGG ATT GGA CAT GAT GCT GGG CAT TAT Phe Leu Trp Ile Gln Ser Gly Trp Ile Gly His Asp Ala Gly His Tyr 150 155 160	534
ATG GTA GTG TCT GAT TCA AGG CTT AAT AAG TTT ATG GGT ATT TTT GCT Met Val Val Ser Asp Ser Arg Leu Asn Lys Phe Met Gly Ile Phe Ala 165 170 175 180	582
GCA AAT TGT CTT TCA GGA ATA AGT ATT GGT TGG TGG AAA TGG AAC CAT Ala Asn Cys Leu Ser Gly Ile Ser Ile Gly Trp Trp Lys Trp Asn His 185 190 195	630
AAT GCA CAT CAC ATT GCC TGT AAT AGC CTT GAA TAT GAC CCT GAT TTA Asn Ala His His Ile Ala Cys Asn Ser Leu Glu Tyr Asp Pro Asp Leu 200 205 210	678
CAA TAT ATA CCA TTC CTT GTT GTG TCT TCC AAG TTT TTT GGT TCA CTC Gln Tyr Ile Pro Phe Leu Val Val Ser Ser Lys Phe Phe Gly Ser Leu 215 220 225	726
ACC TCT CAT TTC TAT GAG AAA AGG TTG ACT TTT GAC TCT TTA TCA AGA Thr Ser His Phe Tyr Glu Lys Arg Leu Thr Phe Asp Ser Leu Ser Arg 230 235 240	774
TTC TTT GTA AGT TAT CAA CAT TGG ACA TTT TAC CCT ATT ATG TGT GCT Phe Phe Val Ser Tyr Gln His Trp Thr Phe Tyr Pro Ile Met Cys Ala 245 250 255 260	822
GCT AGG CTC AAT ATG TAT GTA CAA TCT CTC ATA ATG TTG TTG ACC AAG Ala Arg Leu Asn Met Tyr Val Gln Ser Leu Ile Met Leu Leu Thr Lys 265 270 275	870
AGA AAT GTG TCC TAT CGA GCT CAG GAA CTC TTG GGA TGC CTA GTG TTC Arg Asn Val Ser Tyr Arg Ala Gln Glu Leu Leu Gly Cys Leu Val Phe 280 285 290	918
TCG ATT TGG TAC CCG TTG CTT GTT TCT TGT TTG CCT AAT TGG GGT GAA Ser Ile Trp Tyr Pro Leu Leu Val Ser Cys Leu Pro Asn Trp Gly Glu 295 300 305	966
AGA ATT ATG TTT GTT ATT GCA AGT TTA TCA GTG ACT GGA ATG CAA CAA Arg Ile Met Phe Val Ile Ala Ser Leu Ser Val Thr Gly Met Gln Gln 310 315 320	1014

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GTT CAG TTC TCC TTG AAC CAC TTC TCT TCA AGT GTT TAT GTT GGA AAG Val Gln Phe Ser Leu Asn His Phe Ser Ser Val Tyr Val Gly Lys 325 330 335 340	1062
CCT AAA GGG AAT AAT TGG TTT GAG AAA CAA ACG GAT GGG ACA CTT GAC Pro Lys Gly Asn Asn Trp Phe Glu Lys Gln Thr Asp Gly Thr Leu Asp 345 350 355	1110
ATT TCT TGT CCT CCT TGG ATG GAT TGG TTT CAT GGT GGA TTG CAA TTC Ile Ser Cys Pro Pro Trp Met Asp Trp Phe His Gly Gly Leu Gln Phe 360 365 370	1158
CAA ATT GAG CAT CAT TTG TTT CCC AAG ATG CCT AGA TGC AAC CTT AGG Gln Ile Glu His His Leu Phe Pro Lys Met Pro Arg Cys Asn Leu Arg 375 380 385	1206
AAA ATC TCG CCC TAC GTG ATC GAG TTA TGC AAG AAA CAT AAT TTG CCT Lys Ile Ser Pro Tyr Val Ile Glu Leu Cys Lys Lys His Asn Leu Pro 390 395 400	1254
TAC AAT TAT GCA TCT TTC TCC AAG GCC AAT GAA ATG ACA CTC AGA ACA Tyr Asn Tyr Ala Ser Phe Ser Lys Ala Asn Glu Met Thr Leu Arg Thr 405 410 415 420	1302
TTG AGG AAC ACA GCA TTG CAG GCT AGG GAT ATA ACC AAG CCG CTC CCG Leu Arg Asn Thr Ala Leu Gln Ala Arg Asp Ile Thr Lys Pro Leu Pro 425 430 435	1350
AAG AAT TTG GTA TGG GAA GCT CTT CAC ACT CAT GGT T AAAATTACCC Lys Asn Leu Val Trp Glu Ala Leu His Thr His Gly 440 445	1397
TTAGTTCATG TAATAATTTG AGATTATGTA TCTCCTATGT TTGTGTCTTG TCTTGTTCT	1457
ACTTGTTGGA GTCATTGCAA CTTGTCTTTT ATGGTTTATT AGATGTTTTT TAATATATTT	1517
TAGAGGTTTT GCTTTCATCT CCATTATTGA TGAATAAGGA GTTGCAATATT GTCAATTGTT	1577
GTGCTCAATA TCTGATATTT TGGAATGTAC TTTGTACCAC GTGGTTTTCA GTTGAAGCTC	1637
ATGTGTACTT CTATAGACTT TGTTTAAATG GTTATGTCAT GTTATTT	1684

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 843 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GAATTCTATC ACTAGTGACC ACCCCATCCC CTTATTTCAA TAATGGAACA CAAAAAATT	60
TTAAAAAATA GTTGCTGTTA ATTGTTTAAC CGTCATTTTC CAACATTACT AGCTAATCGT	120
TAATTGATCT TCATAAAAAA AAAAATTGCT ATGGGTACTA TTGAGATTGT ATATCTTATC	180
AGTTAGGCCT AAGGGGGGGG TCAGTGATAT TACGAATGAT ACAAACATCA ACGCGTGGAA	240
CATTACAAAA TTCCGTAATT TTTTCAACGC CGTGATGGTT TTTTTTTTTT TTTTTTTTTT	300
TGATGGTAAT TGTGTTGG GGGGAAATTA TTGGGTATGG TGTGAGTAT GACCACCCCC	360
ACTAAAAAAG GTTGTGAGTG ATGTAAAAAT GGTGCTGAC ATGACGAAAC ATAATTGGAT	420
ATTGTGAGTG ATAAAAATTT ATCATTAGTG ACCACCCCGC CTCCCCTTAT CATATGTTGT	480
TATCTTCCAT AGTTGCGGTA TACCAACATA TGGTAGTTTT TATATTTATA GTTTATATTT	540
TCATTAACT CTCTTCGCCA GGCTACTTGT ATTGTAATCA TATGGAATCT CAACTCCAGT	600
TGGAGCCATT CCATCATATA TTTCCATTTC CAAACAAAGA GAATTGACAC CTCATACATA	660
CTCCAAAGCA TACTTCCACT TGCTATAATT TTCATGTAAA AACTCGTACG TGTTATTCGA	720
CAATGTTTAT ATAACGCCAC CGATTAACT CACCTCTCCA CGTATGAACC TCCACCCACC	780
ATATATACGC ACCACCACCA CACCATAATT CACACAACCA CAACACCATC TCCCACAGGA	840
TCC	843

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Trp	Ile	Gly	His	Asp	Ala	Gly	His
1							5

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Asn Val Gly His Asp Ala Asn His
1 5

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Val Leu Gly His Asp Cys Gly His
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Val Ile Ala His Glu Cys Gly His
1 5

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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Val Ile Gly His Asp Cys Ala His
1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Val Val Gly His Asp Cys Gly His
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

His Asn Ala His His
1 5

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(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

His Asn Tyr Leu His His
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

His Arg Thr His His
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

His Arg Arg His His
1 5

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(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

His Asp Arg His His
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

His Asp Gln His His
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

His Asp His His His
1 5

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(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

His Asn His His His
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Phe Gln Ile Glu His His
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

His Gln Val Thr His His
1 5

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(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

His Gln Val Thr His His
1 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

His Val Ala His His
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

His Ile Pro His His
1 5

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(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

His Val Pro His His
1 5

What is claimed is:

1

1. An isolated nucleic acid encoding an albumin 5' regulatory region which directs seed-specific expression selected from the group consisting of the nucleotide sequence set forth in SEQ ID NO:2, the nucleotide sequence set forth in SEQ ID NO:2 having an insertion, deletion, or substitution of one or more nucleotides, and a contiguous fragment of the nucleotide sequence set forth in SEQ ID NO:2.

10

2. An expression cassette which comprises the albumin 5' regulatory region of Claim 1 operably linked to a heterologous gene.

3. The expression cassette of Claim 2 wherein the heterologous gene is at least one of a fatty acid synthesis gene or a lipid metabolism gene.

15

4. The expression cassette of Claim 3 wherein the heterologous gene is selected from the group consisting of a lipid desaturase gene, an acyl carrier protein (ACP) gene, a thioesterase gene, an acetyl transacylase gene, an acetyl-coA carboxylase gene, a ketoacyl synthase gene, a malonyl transacylase gene or an elongase gene.

20

5. The expression cassette of Claim 4 wherein the lipid desaturase gene is selected from the group consisting of a $\Delta 6$ -desaturase gene, a $\Delta 12$ -desaturase gene, and a $\Delta 15$ -desaturase gene.

25

6. An expression vector which comprises the expression cassette of any one of Claims 2-5.

7. A cell comprising the expression cassette of any one of Claims 2-5.

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8. A cell comprising the expression vector
1 of Claim 6.

9. The cell of Claim 7 wherein said cell is
a bacterial cell or a plant cell.

10. The cell of Claim 8 wherein said cell
5 is a bacterial cell or a plant cell.

11. A transgenic plant comprising the
expression cassette of any one of Claims 2-5.

12. A transgenic plant comprising the
expression vector of Claim 6.

13. A plant which has been regenerated from
10 the plant cell of Claim 9.

14. A plant which has been regenerated from
the plant cell of Claim 10.

15. The plant of Claim 12 or 13 wherein
said plant is at least one of a sunflower, soybean,
maize, cotton, tobacco, peanut, oil seed rape or
Arabidopsis plant.

16. Progeny of the plant of Claim 11 or 12.

17. Seed from the plant of Claim 11 or 12.

18. A method of producing a plant with
20 increased levels of a product of a lipid metabolism
gene which comprises:

(a) transforming a plant cell with an
expression vector comprising the isolated nucleic acid
25 of Claim 1 operably linked to at least one of an
isolated nucleic acid coding for a fatty acid
synthesis gene or a lipid metabolism gene; and

(b) regenerating a plant with increased
levels of the product of said fatty acid synthesis

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gene or said lipid metabolism gene from said plant
1 cell.

19. A method of producing a plant with
increased levels of gamma linolenic acid (GLA) content
which comprises:

5 (a) transforming a plant cell with an
expression vector comprising the isolated nucleic acid
of Claim 1 operably linked to a $\Delta 6$ -desaturase gene;
and

(b) regenerating a plant with increased
10 levels of GLA from said plant cell.

20. The method of Claim 19 wherein said $\Delta 6$ -
desaturase gene is at least one of a cyanobacterial
 $\Delta 6$ -desaturase gene or a borage $\Delta 6$ -desaturase gene.

21. The method of Claim 18 or 19 wherein
15 said plant is a sunflower, soybean, maize, tobacco,
cotton, peanut, oil seed rape or *Arabidopsis* plant.

22. The method of Claim 18 wherein said
fatty acid synthesis gene or said lipid metabolism
gene is at least one of a lipid desaturase, an acyl
20 carrier protein (ACP) gene, a thioesterase gene an
elongase gene, an acetyl transacylase gene, an acetyl-
CoA carboxylase gene, a ketoacyl synthase gene, or a
malonyl transacylase gene.

23. A method of inducing production of at
25 least one of gamma linolenic acid (GLA) or
octadecatetraenoic acid (OTA) in a plant deficient or
lacking in GLA which comprises transforming said plant
with an expression vector comprising an the isolated
nucleic acid of Claim 1 operably linked to a $\Delta 6$ -
30

desaturase gene and regenerating a plant with
1 increased levels of at least one of GLA or OTA.

24. A method of decreasing production of a
fatty acid synthesis or lipid metabolism gene in a
plant which comprises:

- 5 (a) transforming a plant cell with an
expression vector comprising the isolated nucleic acid
of Claim 1 operably linked to a nucleic acid sequence
complementary to a fatty acid synthesis or lipid
metabolism gene; and
10 (b) regenerating a plant with decreased
production of said fatty acid synthesis or said lipid
metabolism gene.

25. A method of cosuppressing a native
fatty acid synthesis or lipid metabolism gene in a
15 plant which comprises:

- (a) transforming a cell of the plant with an
expression vector comprising the isolated nucleic acid
of Claim 1 operably linked to a nucleic acid sequence
encoding a fatty acid synthesis or lipid metabolism
20 gene native to the plant; and
(b) regenerating a plant with decreased
production of said fatty acid synthesis or said lipid
metabolism gene.

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35

3
ata tct gcc tac cct ccc aaa gag agt agt cat ttt tca tca atg gct gct caa atc aag
H A A O I K
62
aaa tac att acc tca gct gaa ctc aag aac cac gat aaa ccc gga gat cta tgg acc tgg
K Y I T S U R L K H H D K P G D L M I S
122
att caa ggg aaa gcc tat gat gtt tgg gat tgg gtc aaa gac cat cca ggt ggc agc ttt
I O G K A Y D V S D W V K D H P O G S F
182
ccc tgg aag agt ctt gct ggt caa gag gta act gat gca ttt gtt gca ttc cat cct gcc
P L K S L A G O E V T D A F V A F H P A
242
tct acc tgg aag aat ctt gat aag ttt ttc act ggg tat tat ctt aaa gat tac tct gtt
S T W K H I D K F F T G Y Y L K D Y S V
302
tct ggg gtt tct aaa gat tat aag aag cct gtc ttt gag ttt tct aaa atg ggt ttg tct
S E V S K D Y R K L V F E F S K H G L Y
362
gac aaa aaa ggt cat att atg ttt gca act ttg tgg ttt ata gca atg ctg ttt gct atg
D R K G H I H F A T L C F I A H L F A H
422
agt gtt tat ggg gtt ttg ttt tgt gag ggt gtt ttg gca cat ttg ttt tct ggg ttt tgg
S V Y G V L F C E G V L V H L F S G C L
482
atg ggg ttt ctt tgg att cag agt ggt ggt att gga cat gat gct ggg cat tat atg gca
M O P L M I O S O M Y O H D A D H Y H V
542
gtg tct gat tca agg ctt aat aag ttt atg ggt att ttt gct gca aat tgt ctt tca gaa
V S D S R I H K P M G I F A A N C L S G
602
ata agt att ggt tgg tgg aaa tgg aac cat aat gca cat cac att gcc tgt aat agc ctt
I S I O W M K W H H A H H I A C H S L
662
gaa tat gac cct gat tta caa tat ata cca ttc ctt gtt gtc tct tca aag ttt ttt ggt
R Y D P D L O Y I P F L V V S S K F F G
722
tca ctc acc tct cat ttc tat gag aaa aag ttg act ttt gac tct tta tca aga ttc ttt
S L T S H F Y E K R L T F D S L S R F F
782
gta agt tat caa cat tgg aca ttt tcc att atg tgt gct gct agg ctc aat atg tat
V S Y O H W T F Y P I H C A A R L H H Y
842
gta caa tct ctc ata atg ttg ttg acc aag aga aat gtc tcc tat cga gct cag gaa ctc
V O S L I H I L I T K R H V S Y P A O E L
902
ttg gga tgc cta gtc ttc tgg att tgg tcc cgg ttg ctt gtt tct ttt ttg cct aat tgg
L G C L V F S I M Y P L L V S C L P H W
962
ggt gaa aga att atg ttt gtt att gca agt tta tca gtc act gga atg caa caa gtt cag
O S R I H P V I A S L S V T G H O Q V O
1022
tto tcc ttg aac cao ttc tct tca agt gtt tat gtt gga aag cct aaa ggg aat aat tgg
F S L H H F S S S V Y V G K P K O H H W
1082
ttt gag aaa caa ccg gat ggg aca ctt gac att tct tct cct cct tgg atg gat tgg ttt
F E K O T D G T L D I S C P P W H D W F
1142
cat ggt gga ttg caa ttc caa att gag cat cat ttg ttt ccc aag atg cct aga tgg aac
H O G L O F O I P H H L F P K H P R C H
1202
ctt ggg aaa atc tgg ccc tac gtc atc ggg tta tcc aag aaa cat aat ttg ctt tcc aat
L R K I S P Y V I E L C K K H H L P Y H
1262
tat gca tct ttc tcc aag gcc aat gaa atg aca ctc aga aca ttg agg aac aca gaa ttg
Y A S F S K A H E M T L R T L R H T A L
1322
cag gct agg gat ata acc aag ccg ctc ccg aag aat ttg gta tgg gaa gct ctt cag act
O A R D I T K P L P K N L V W S A L H T
1382
cat ggt taa aat tac cct tgg ttc atg taa taa ttt gag att atg tat ctc cta tgt ttg
H G
1442
tgt ctt gtc ttg gtt cta ctt gtt gga gtc att gca act tgt ctt tta tgg ttt att aga
1502
tgt ttt tta ata tat ttt aga ggt ttt gct ttc acc tcc att att gat gaa taa gga gtt
1562
gca tat tgt caa ttg ttg tgc tca ata tct gat acc ttg gaa tgt act ttg tcc cag tgt
1622
ggt ttc agt tga agc tca tgc gaa ctt cta tgg act ttg ttt aaa tgg tta tgt cat gtt
1682
acc t

FIGURE 1

FIGURE 2

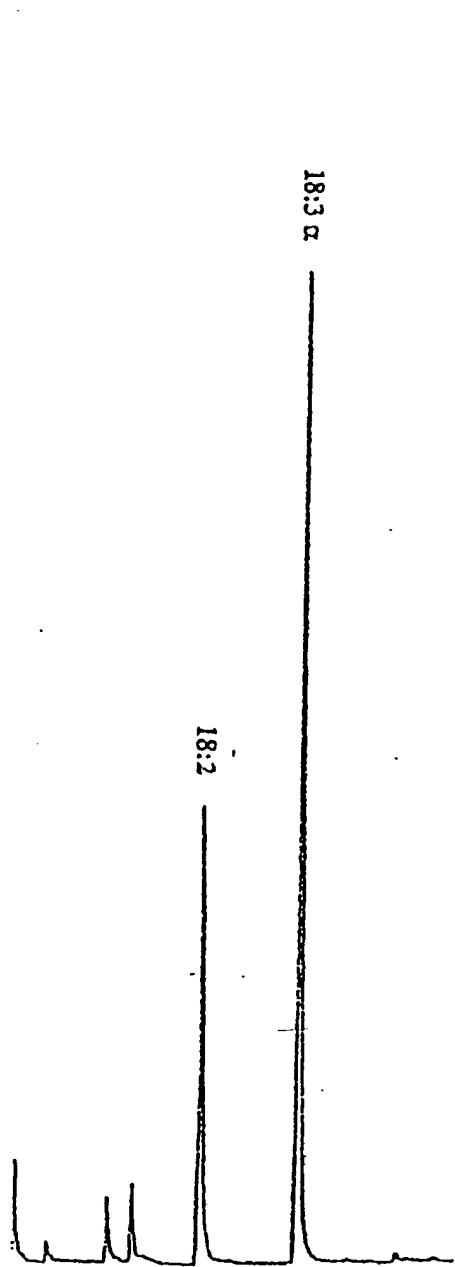


FIGURE 3A

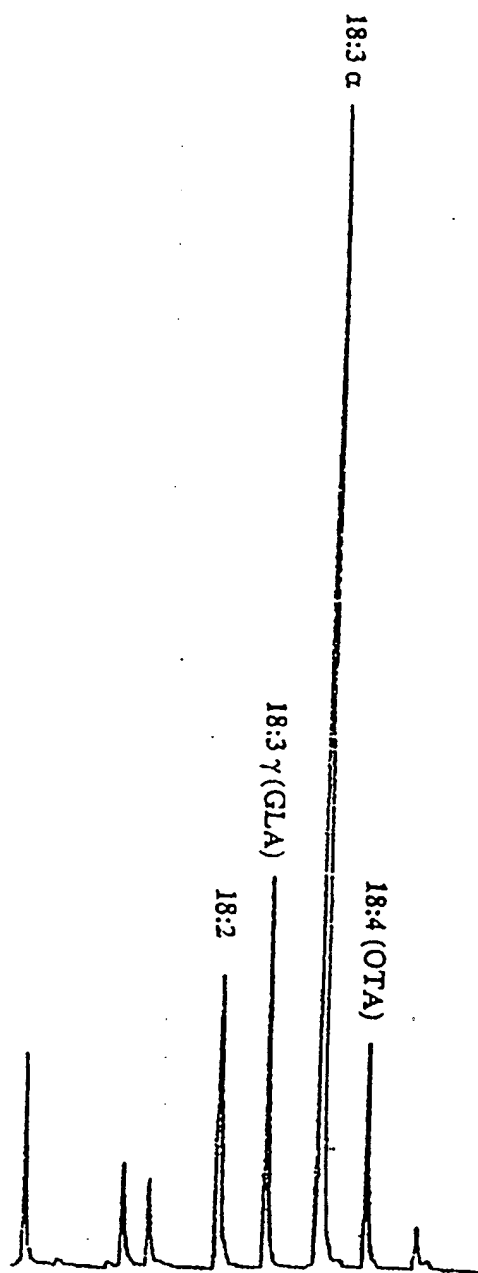


FIGURE 3B

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GAAATCTATC ACTAGTGACC ACCCCATCCC CTATATTTCAA TAAATGGACA -811
CAAAAAAAAAAT TTTAAAAAAT AGTTCGTGT AATGTGTTAA CCGTCATTTT -761
CCAACATTAC TAGCTAATCG TTAAATTGATC TTCAFAAAA AAAAAATTCG -711
TATGGGTAAT ATTGAGATIG TATATCTTAT CAGTTAGGCC TAAGGGGGCG -661
GTCAGIGATA TTACGAATGA TACAAACATC AACGGGIGGA ACATTACAAA -611
TTCCTATCCC CACCTCCAAG TATAACGGGT GTTGTGTCCA CGGTGTGATG -561
ATTCGGTAAT TTTTCAACG CCGTATGGT TTTTTTTTTT TTTTITTTT -511
TTGATGGTAA TGTGTGTG GGGGGAAAT ATGGGTATG GGTGTGAGTG -461
ATGACCACCC CCACFAAAA AGGTGTGTGAG TGAATGAAA ATGGTGTCTG -411
ACATGACGAA ACATTAATGG ATATGTGTGAG TGATAAAAT TTATCATATG -361
TGACCACCCC GCCTCCCCIT ATCATATGTT GTTATCTTCC ATAGTGTCCG -311
TATACCAACA TATGGTAGTT TTTATATTA TAGTGTATAT TTTCATFAAA -261
CTCTCTTCCG CAGGCTACTT GTATTGTAA CATAATGGAAT CTCAACTCCA -211
CTTGGAGCCA TTCCATCAT TATTTCATT TCCAAACAAA GAGAAITGAC -161
ACCICATACA TACITCAAAG CATACTTCCA CTGTCTATAA TTITICATGA -111
AAACITCGTA CGTGTATAT GACAATGTTC ATATAACGCC ACCGATFAAA -61
CTCACCITCT CACGTATGAA CCTCCACCCA CCATATATAC GCACCACCAC -11
CACCCATFA TACACACAAC CACAACCCA TCTCCACAG GATCC -29

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FIGURE 4

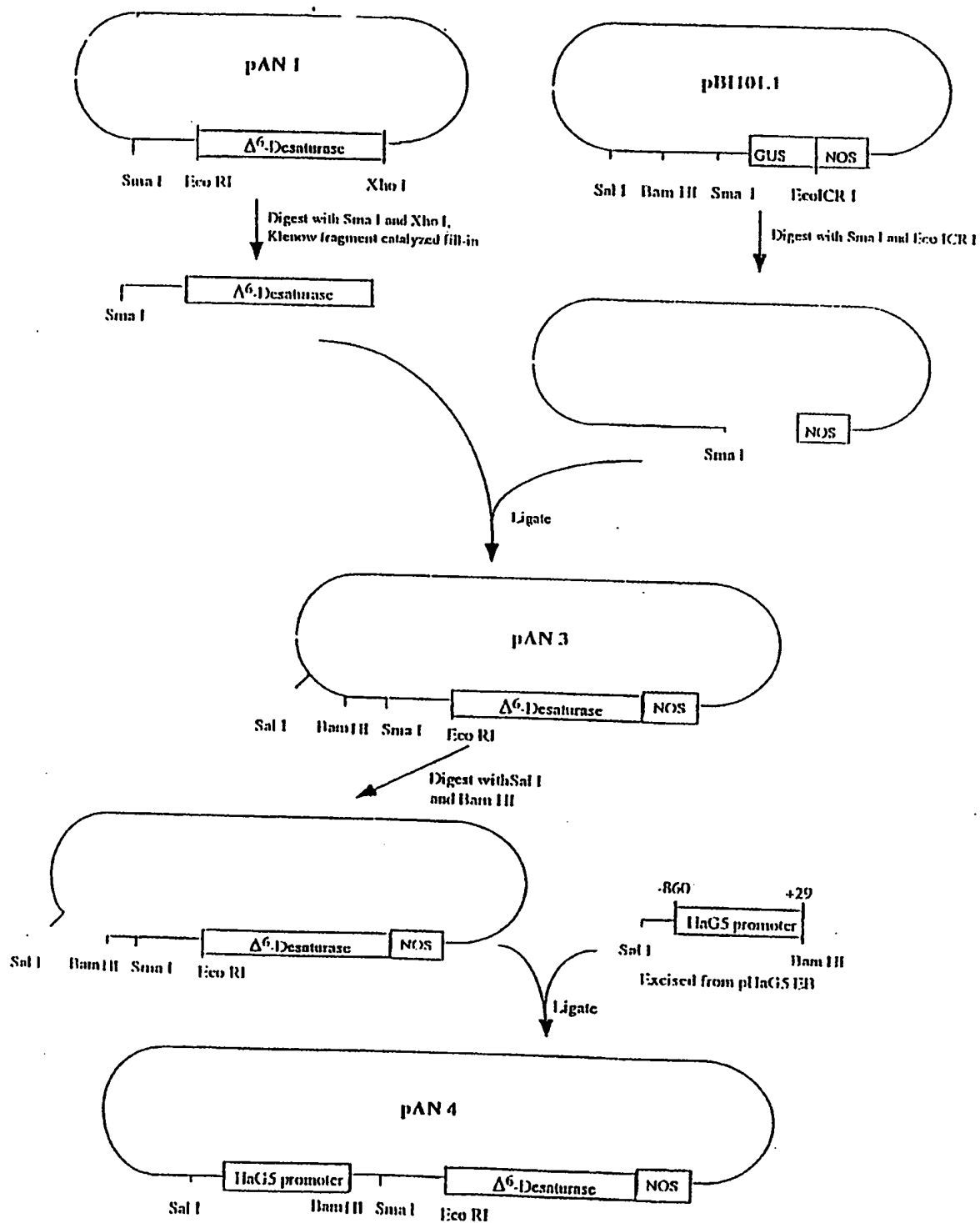
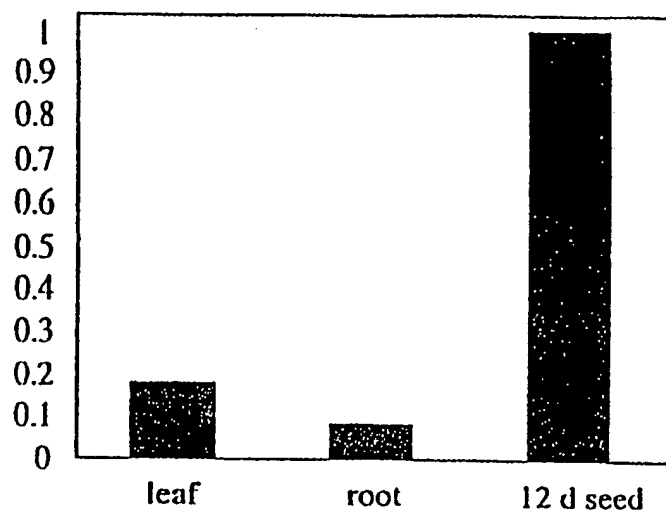


FIGURE 6B

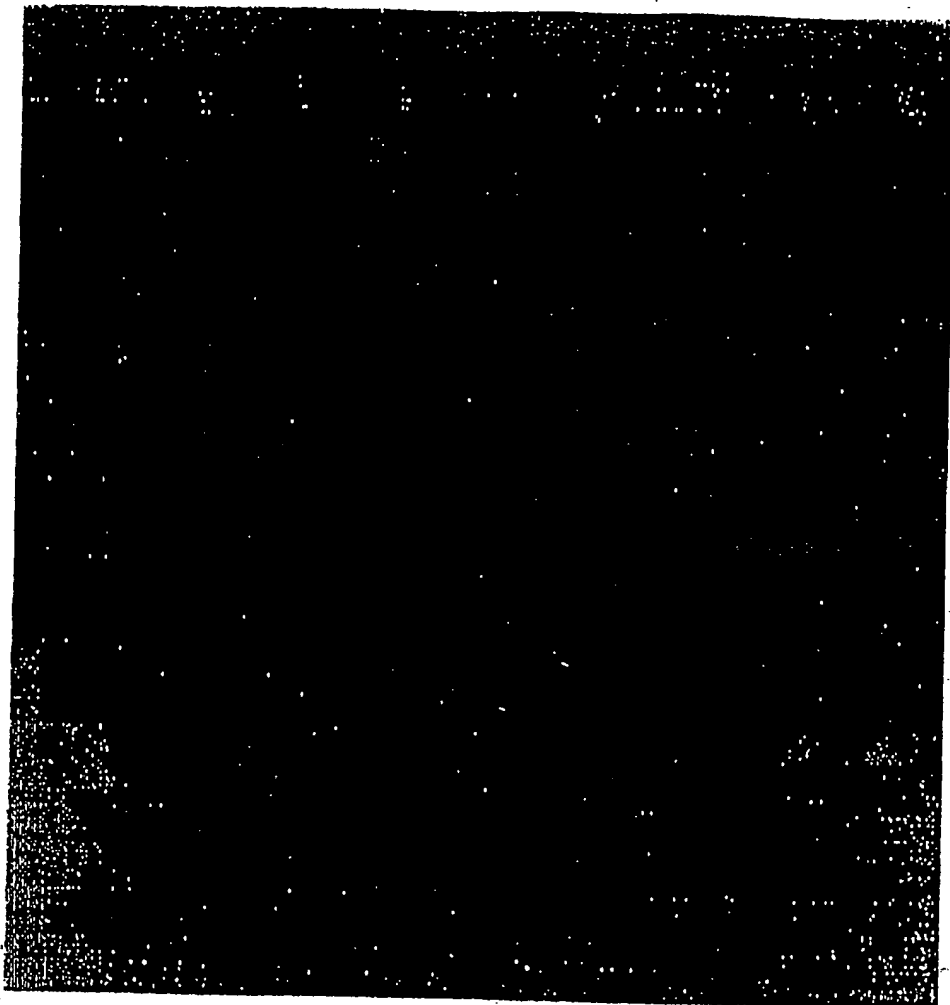


Borage tissue



FIGURE 6A

FIGURE 7



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/07178

A. CLASSIFICATION OF SUBJECT MATTER		
IPC 6	C12N15/82	C12N15/29 C12N15/53 A01H5/00 A01H5/10
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
IPC 6 C12N A01H		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
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A	--- WO 94 10189 A (CALGENE INC) 11 May 1994 see examples 3,4	1-4
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<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family		
Date of the actual completion of the international search		Date of mailing of the international search report
4 September 1998		07.09.98
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Maddox, A

INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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A	WO 92 11373 A (DU PONT) 9 July 1992 see page 67, line 25 - page 68, line 13 ---	1-4
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A	WO 94 11516 A (DU PONT ;LIGHTNER JONATHAN EDWARD (US); OKULEY JOHN JOSEPH (US)) 26 May 1994 see page 98 - page 110 ---	1,3-5,24
A	WO 96 06936 A (DU PONT ;HITZ WILLIAM DEAN (US)) 7 March 1996 see page 48, line 23 - page 53, line 10 ---	1-5,24, 25
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